



iPS Cell Generation Kit

Cat. Numbers:

ASK-3011
ASR-2000

ASR-2001
ASR-2002

ASR-2003
ASR-2004

Kit Contents

Component	Size	Storage
VSVg GFP retrovirus	1 ml	-80°C
VSVg hOct4 retrovirus	1 ml	-80°C
VSVg hSox2 retrovirus	1 ml	-80°C
VSVg hKlf4 retrovirus	1 ml	-80°C
VSVg hL-Myc retrovirus	1 ml	-80°C
Polybrene		-20°C
Reprogramming Media		-20°C

Materials not included in the kit

- Coating matrix
- Feeder cells

Special Handling

1. VSVg retrovirus should be stored at -80°C.
2. Repeated freeze/thaw may decrease titer. Making aliquots after first thaw is strongly recommended.
3. Handling VSVg retrovirus should be performed in a BSL-2 lab.

Before you begin reprogramming

NOTE: Transduction efficiency varies in different cell types. Initial optimization may be needed to determine MOI value used for reprogramming.

1. Seed the cells that will be reprogrammed at a density of 4×10^4 cells per well on a 24-well plate.
2. Incubate the 24-well plate at 37°C / 5% CO₂ overnight.
3. Thaw one vial of GFP retrovirus at room temperature. While thawing, label four Eppendorf tubes: 10 ul, 40 ul, 160 ul and 480 ul.
4. Aliquot the appropriate volume of GFP retrovirus (10 ul, 40 ul, 160 ul, 480 ul) to the corresponding Eppendorf tube. Adjust the volume of each vial to 500 ul and add polybrene to a final concentration to 5 ug/ml.
5. Add the above GFP retrovirus samples to the 24-well plate prepared in step 1.

6. Incubate at 37°C / 5% CO₂ overnight.
7. Change the media to the original media for the continuous culture
8. Determine GFP expression level 48-72 hours post-transduction by flowcytometry
9. Calculate the MOI based on the well that has 20-50% GFP expression.
MOI = the volume of the virus used (ml) x virus titer (IU/ml) / cell number

Sample calculation:

The volume of the virus used: 0.16 ml
Virus titer (shown in spec sheet): 3.79×10^5 IU/ml
Cell number: 4×10^4
MOI = (0.16 ml) x (3.79×10^5 IU/ml) / (4×10^4) = 1.516

Reprogramming (iPS generation)

Day 1-2

1. Plate 2×10^6 cells on a 10 cm tissue culture dish containing 10 ml of cell culture media. Incubate at 37°C / 5% CO₂ for 2 days. Change media after 24 hours.
NOTE: Best results are obtained using early-passaged cells.

Day 3

2. Prepare a 6-well plate by adding 2 ml of 0.1% Gelatin Type A to each well.
3. Remove the cell culture media from the plate prepared in step 1.
4. Wash the cells 1-2 times with PBS.
5. Add 2 ml TrypLE and incubate at 37°C for 5 minutes.
6. Add 5 ml of cell culture media to neutralize TrypLE.
7. Transfer the cells to a sterile 50 ml conical tube.
8. Remove a small aliquot to determine the cell density.
9. Centrifuge the 50 ml conical tube at 1,000 rpm for 5 minutes.
10. Carefully remove the supernatant from the 50 ml conical tube and add a predetermined volume (using the cell density determined in step 8.) of cell culture media to make a cell suspension of 5×10^4 cells/ml.
11. Aspirate the gelatin solution from the 6-well plate prepared in step 2.
12. Add 2 ml of the cells to each well.
13. Gently distribute the cells evenly in the wells and incubate the 6-well plate at 37°C / 5% CO₂ overnight.

DAY 4

14. Calculate the volume of each virus to the MOI determined in the initial optimization step.
NOTE: Titers vary between each lot. Check the titers shown in the spec sheets of each lot.
Virus volume (ml) = cell number x MOI / virus titer

Sample Calculation:

Cell number: 1×10^5
Virus titer (shown in spec sheet): 9.47×10^5 IU/ml
MOI: 1.516
Virus volume (ml) = (1×10^5) x 1.516 / (9.47×10^5) = 0.160 ml

15. Thaw the retrovirus at room temperature.
16. Take out the volume needed for transduction, and put the remaining virus back at -80°C.

PROTOCOL

Note: Repeated freeze/thaw may decrease the virus titer. Aliquoting all four viruses after the first thaw is highly recommended.

17. Mix the viruses together, adjust the volume to 3 ml with culture media, and add polybrene to a final concentration of 5 ug/ml

Note: Co-transduction of VSVg GFP retrovirus with other retrovirus can be an easy way to monitor the transduction efficiency by GFP expression, and the reprogramming status by GFP silencing.

18. Remove the media from the cells from the 6-well plate in step 13.
19. Add the virus mixture to each well where the cells are designated to be reprogrammed.
20. Add culture media to the wells where the cells are designated as controls.

Optional: For transduction controls, add only GFP retrovirus on the well.

DAY 5 (Optional)

21. Incubate at 37°C / 5% CO₂ overnight.
22. Wash the cells 3 times with cell media and then add 2 ml media/well.
23. Incubate at 37°C / 5% CO₂ overnight.
24. Repeat steps 18-23.

Note: Repeated transduction may increase reprogramming efficiency. For the cells which have low transduction efficiency, or high toxicity, repeated transduction with a low-MOI of retrovirus is recommended.

DAY 6

25. Remove the virus-containing media
26. Wash 3 times with reprogramming media
27. Add fresh reprogramming media
28. Incubate at 37°C / 5% CO₂ for three days
29. Change media every 2-3 days until iPSC cell colonies can be observed.

CLONE ISOLATION / EXPANSION DAYS 10-14

30. Once iPSC colonies begin to grow, prepare a fresh 12-well MEF plate.
31. Using an 18 gauge needle, Pipettman tips or modified Pasteur pipette, partially dislodge the iPSC colony from the plate surface.
32. Using a P-200 or similar device, transfer the iPSC colony into a well of the 12-well MEF plate. In order to keep track of clones you will screen, be sure to designate each prospective clone with some type of identification system.
33. Repeat steps 29-30 for each prospective clone.
34. Passage each candidate clone two times using freshly made 12-well MEF plates.
NOTE: During these two passages, it is normal that some of the candidate iPSC clones will either differentiate or die off. Only continue to passage candidates that exhibit strong iPSC morphology.
35. Expand each candidate clone to a 6-well plate. Increase your passage ratio with successive passages such that you have enough colonies for: characterization, freezing and experiments.

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